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A REVIEW OF PROTEASE INHIBITORS FROM DIFFERENT SOURCES

K. VIJAYA RACHEL[1] & GANDREDDI V D SIRISHA[2]

[1]Assistant Professor, Department of Biochemistry, Institute of Science, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh, India

[2]Research Scholar, Department of Biochemistry, Institute of Science, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh, India

ABSTRACT

Protease inhibitors (PIs) are widely distributed in all living forms such as microorganisms, plants and animals. Protease inhibitors are involved in regulation of enzyme activity deregulation of which leads to diseases. Protease inhibitors are of many types which are categorized based on the specificity, amino-acid sequences, localization of the reactive sites, disulfide bridge topology, mechanism of action and three-dimensional structure. The different types of Protease inhibitors include aspartate protease inhibitors, serine protease inhibitors, cysteine protease inhibitors and metallo carboxypeptidase protease inhibitors. Protease inhibitors find application in different areas like medicine, agriculture and biotechnology. Specific protease inhibitors are effective tools for inactivating the target proteases which are involved in pathogenic process of human diseases like arthritis, pancreatitis, hepatitis, cancer, AIDS, thrombosis, emphysema, high blood pressure, muscular dystrophy etc. Protease inhibitors are one of the prime molecules with highly proven inhibitory activity against insects, pests and are used as bioinsecticides by developing transgenic plants. A brief review of isolation, identification, characterization and applications of various Protease inhibitors is carried out.

KEYWORDS: Protease Inhibitors, Classification, Target Proteases, Effective Tools, Review

ABBREVIATIONS

PIs	-	Protease Inhibitors
PR	-	Pathogenesis related
APIs	-	Aspartic protease Inhibitors
SPIs	-	Serine Protease inhibitors
CPIs	-	Cysteine protease inhibitors
MCPIs	-	Metallo carboxyprotease inhibitors.

INTRODUCTION

Plants and animals have developed defense mechanisms against pathogenic microorganisms such as viruses, bacteria and fungi. This is by the production of biochemical compounds before or after the pathogen enters into the host body. These compounds may be proteins or other metabolites which inhibit pathogen secreted proteins by different mechanisms. Some of these are pathogenesis-related (PR) proteins called as inhibitors and inhibit the proteolytic enzyme. So these are also termed as protease inhibitors. Protease inhibitors (PIs) are proteins that form stoichiometric high affinity

complexes with proteases and inhibit their hydrolytic activity (Jin*et al.*,2009). Most PIs interact with target proteases by binding to the active site of the protease resulting in the formation of protease-inhibitor complex and is incapable of enzymatic activity (Syed Rakashanda*et al.*, 2012). Proteases regulate activation, synthesis and turnover of all proteins. Uncontrolled proteolysis is deleterious to cellular functions. Nature has developed checkpoints called protease inhibitors as one of the strategies to control them. Uncontrolled proteolysis is implicated in diseases like emphysema, systemic inflammatory response syndrome, arthritis pancreatitis, hepatitis etc (Catherine 2009).

PIs act as defense proteins against insects and microorganisms and possess antibiotic activity against fungi and some viruses (Satheeshand Murugan2011). These inhibitors are targeted against hydrolytic enzymes of microbes and thus prevent microbial growth and its growth. Some natural components (fungal and bacterial proteins, polysaccharides, lipoproteins, viral coat proteins) and synthetic components (salicylic acid, polyacrylic acid and chloroethylphosphonic acid) induce disease resistance in the plants (Agrios 1997). PIs from plant extract acts as lead compounds in synthesis of antimicrobial agents as they can potently inhibit pathogenic microbes (Jin*et al.*, 2009).

Recent developments in the field of plant engineering are to alter the plants towards diseases and insect resistance. For the development of insect resistance crop plants, the role of plants derived PIs was recognized early and resistant transgenic tobacco plants were first reported in 1987 by Hilder. Inhibitory proteins are transferred to tobacco, potato, rice, wheat, cauliflower, pea etc by using Recombinant DNA (rDNA) technology which develops resistance towards insects, fungal and viral pathogens. Different PIs are used to develop insect resistant transgenic plants. These transgenic plants express the transferred insecticidal proteins and enhance the insect resistance. Different PIs that have been used for developing insect- resistant transgenic plants are potato serine PIs, sweet potato PIs, cowpea serine PIs, rice cysteine PIs, soybean kunitz PIs, tobacco PIs, corn cystatins, mustard trypsin inhibitors and bean α- amylase inhibitors. The first PI gene isolated from cowpea was transferred to tobacco plant which resulted in transgenic plant carrying CPTI gene that enhance the *Manduca sexta*(Ussuf*et al.*, 2001).

The PIs are small peptides with low molecular mass and widely distributed in plants, animals and microorganisms (Lingaraju and Gowda 2008). Earlier studies on the distribution pattern of protease inhibitors among seeds of leguminous trees showed an evolutionary relationship between the protease inhibitor family and the legume sub-families (Norioka *et al.*, 1988). Currently, 59 distinct families of protease inhibitors have been recognized. On the basis of amino-acid sequences, localization of the reactive sites, disulfide bridge topology, mechanism of action, three-dimensional structure, and stability to heat and denaturing agent, they have been placed under seven distinct families: Kunitz, Bowman–Birk, Potato inhibitors, Squash, Cereal and Mustard (Birk 2003)

On the basis of sequence homologies of their inhibitor domains, PIs have been classified into 48 families (Rawlings 2012). Proteins containing a single inhibitor unit are termed simple inhibitors, and those that contain multiple inhibitor units are termed complex inhibitors. A total of 11 families belong to the latter category and contain between 2-15 inhibitory domains. Most of these are homotypic, containing inhibitor units from a single family, some are however heterotypic and contain inhibitor unit from different families (Trexler *et al.*,2002).

On the basis of sequence homologies, Kunitz-type protease inhibitors can bind and inhibit serine, cysteine and aspartyl proteases (Oliva *et al.*, 2010). The first inhibitor of this family (SBTI) was obtained from *Glycine max* and other inhibitors have been purified and their primary structures determined. This leadsto the conclusion that these inhibitors are not restricted only to the leguminous group, but are also found in other plants (Birk 2003). Moreover, Kunitz-type

inhibitors reversibly interact with enzyme targets, forming stable complexes influencing their catalytic activities in competitive and non-competitive ways (Migliolo *et al.*,2010). Kunitz-type inhibitors are characterized by molecular masses around 20 kDa, a low cysteine content forming two disulphide bonds, devoid of a-helix and a common structural fold composed of a β-trefoil formed by 12 antiparallel β -strands with long interconnecting loops, presenting one reactive site (single headed) for serine proteinases (Khamrui *et al.,* 2005). Two reactive site (double-headed) Kunitz-type inhibitors have also been described (Azarkan *et al.,* 2008). Structures of enzyme inhibitor complexes provide relevant information for understanding interaction mechanisms (Laskowski and Qasim 2000). The reactive site of Kunitz trypsin inhibitors usually are found to have Lys or Arg, as it occurs in the inhibitors from *Acacia confuse*(Hung *et al.*,1992), *Cassia obtusifolia* (Liao *et al.*,2007), *Enterolobium contortisiliquum* trypsin inhibitor (ECTI) (Batista *et al.,* 1996) and *Leucaena leucocephala* (LITI) (Zhou *et al.,* 2013**).**

The information on plant protease inhibitors can be obtained from web resources like PLANT-PI database (http://www.plantpis.ba.itb.cnr.it/) that provides information on plant protease inhibitors (PIs) and their related genes. The summary for the functional properties of each PI is provided by linking to other sequence databases. From around 129 different plant species, 495 inhibitors and several isoinhibitors are identified and this information is present in PLANT-PI database version released in July 2002 by De Leo *et al*. It also provides other information for each entry on inhibited proteases, its reactive sites, TransPlant expression, heterologous expression and mutational analysis. The sequence information for this database are retrieved from the analysis of literature and of sequences from EMBL and Swiss-PROT databases by means of sequence retrieval service (SRS) at European Bioinformatics institute (EBI, http://srs.ebi.ac.uk). To develop this database the information is taken up from other web resources like MEROPS (http://merops.sanger.ac.uk/) (Rawlings*et al.,* 2008).

The information on proteolytic enzymes, their inhibitors and substrates for PLANT-PI database are retrieved from MEROPS database. This database provides the information related to nomenclature and classification of proteolytic enzymes and their inhibitors. The classification is hierarchical and is based on the system developed by (Rawlings*et al.,* 2004). MEROPS 9.10 constitutes the information of about 3000 individual peptidases and their inhibitors. This databases provides information for inhibitors sequences, structure, peptidases of model organisms, peptidase substrate and also provides cross references.

Based on the selectivity and inhibitory activity the protease inhibitors are classified or categorized into four classes. The aspartic protease inhibitors (pepstatins), serine protease inhibitors (serpins), cysteine protease inhibitors (cystatins) and metallocarboxy protease inhibitors. Among these the serine protease inhibitor (SPI) family is the largest family (Syed Rakashanda *et al.*,2013). Not only the inhibitory activity, this classification was also considered their control over protein synthesis, turnover and physiological functions such as fertilization, growth, digestion, cell signaling or migration, immune defense, wound healing and disease propagation.

Aspartic Protease Inhibitors (APIs)

Aspartic peptidases are small group of proteolytic enzymes of the pepsin family that share the same catalytic apparatus and usually function in acidic condition. The aspartate protease inhibitor constitute only one family that is kunitz-type family. This family comprises protease inhibitors with a molecular mass of 20-22 kDa and contain two S-S bridges. They inhibit cathepsin D and in some cases Trypsin (Park*et al.,* 2005). Aspartic proteases bind to the substrate of 6-10 amino acid regions which are processed with the aid of two catalytic aspartic acid residues (James et al, 1992) in

the active site. Thus there is usually considerable scope for building inhibitor specificity for a particular aspartic protease by taking advantage of the collective interactions between inhibitor, on both sides of scissile amide bond, and a substantial portion of the substrate binding groove of the enzyme. These proteases have one or more flaps that close down to top of the inhibitor- protease interaction. The scissile amide bond undergoes neuclophilic attack by water molecule, which is activated by deprotonated catalytic aspartic acid residue. The protonated aspartate donates a proton to the amide bond and forms a zwitter ionic intermediate which breaks into peptides. The water molecule binds between the enzyme and inhibitor stretching the peptide bond out of planarity towards a tetrahydral transition state that stabilizes the second water molecule (Chatfield and Brooks,1995) Aspartic protease inhibitor crystal structures are currently available in PDB database for viral proteases (HIV-1, HIV-2, SIV, FIV), cathepsin-D, rennin, chymosin, Penicillopepsin, secreted aspartic protease, pepsin, mucoropepsin, retropepsin, saccharopepsin, rhizopuspepsin and plasmepsin-II.

Aspartic protease inhibitors can be grouped into two categories based on their molecular nature. They are (1) Proteinaceous inhibitors and (2) Low molecular weight inhibitors (Chandravanu Dash et al., 2003).

Proteinaceous inhibitors are rare in the nature and have been described in a few plant species, potato (Keilova *et al.,*1976) tomato (*Solanum lycopersicum*) (Werner *et al.,*1993), wheat (*Tricium aestvum*) (Galleschi *et al.,* 1993)*Vicia sativa*(Roszkowska and Bankowska 1998), *Anchusa strigosa*(Abuereish 1998) and squash-*Cucurbita pepo*(Christeller *et al.,* 1998). A8-KDa aspartic protease inhibitor from yeast was reported to inhibit the activity of protease-A (or) Saccharopepsin enzyme (Phylip*et al*., 2001).

Low molecular weight aspartic proteaseinhibitor pepstatin isolated from various species of Streptomyces, is a specific inhibitor of pepsin (Umezawa*et al.,* 1970). Pepstatins, pepstanones, and hydroxypepstatins have almost identical activity against pepsin and cathepsin D. Pepstatin isalso effective against rennin. Pepstatin inhibits the growth of *Plasmodium beghei* and inhibits murine sarcoma virus (Yuasa *et al.,*1975).

Lycopodium cernuum secretesnatural products like lycermic acid-C and Apigenin-4-0 (2",6"-di-o-P-conmarly) β-D-glucoside which inhibits the Secreted aspartyl proteinase and suppress the candidal infections(Zhang*et al.,* 2002). Andmany other synthetic aspartic protease inhibitors saquinavir (Roberts *et al.,*1990), Ritonavir (Lea and Faulds 1996), Indianvir (Lacy and Abriola 1996), Nelfinavir (Shetty*et al.,* 19960), Amprenavir (Kim*et al.,* 1995), Lopinavir (Carrillo*et al.,*1998) inhibits the HIV-1 Protease enzyme and controls the AIDS. Antifungal protein from black soy bean (*Glycine soja*) potentially inhibits the growth of mycelium of the *Fusarium oxysporum* and *Mycosphaerella arachidicola* (Ng*et al.,* 2000). It also inhibits the HIV – 1 reverse transcriptase. 17 KDa inhibitor of Pepsin and Cathepsin-E from the parasite *Ascaris luumbicoides*(Kageyama, T. 1998), proteins from potato, tomato and squash (Kreft*et al.,* 1997), Christeller *et al.,* 1998)and a pluripotent inhibitor from sea anemone of cysteine peptidase are the examples for the aspartate protease Inhibitors. Aproteinaceous aspartic proteinase inhibitor corresponding to the family of cathepsin D inhibitors (CDIs) was described in potato (*solanum tuberosum*) involving upto 15 iso forms with a high sequence similarity (Lison*et al.,*2006). The two iso forms of CDI are PDI (Protein disulfide isomerase) and NDIwhich shows the inhibitory activity against aspartate proteinases (Keilova and Tomasek 1976). JIP21 (jasmonic-induced protein 21) shows powerful activity as a chymotryspin inhibitor. Tomato plants over expressing JIP21 have been generated and resistance against larvae of the Lepidopteran Egyptain cotton worm (*spodoptera litoralis*) was obtained (Novatus Mushi*et al.,* 2012).

Serine Protease Inhibitors (SPIS)

Serine Protease inhibitors are the largest family of inhibitors distributed throughout nature.Most serine protease inhibitors are low-molecular mass molecules (3–25 kDa) that inhibit trypsin and/or chymotrypsin. Serine protease inhibitors are classified into different categories based on their substrate specificity particularly by the type of residue found in PI as either Trypsin- like(positively charged amino acid residue), elastase- like(small hydro phobic amino acid residue) (or) Chymotrypsin-like(large hydrophobic residue)(Barrett*et al*., 1998).

Recently Serine protease inhibitor LC-PI-Iis isolated from the plant *Lavatera cashmeriana camb* seeds. The LC-PI-I shows strong inhibition on the growth of *Klebsiella pneumonia* and *Pseudomaonas aeuriginosa*(Syed Rakashanda*et al.*, 2012) which cause urinary tract infection, Pneumonia and septicemia in humans.Protease inhibitors also have recently attracted attention because of their potent anti-carcinogenic effect in various in vivo and in vitro systems (Gills*et al.*, 2007). Recent studies have reported that PI's are employed as new drugs in antiretroviral combination therapy whichincreases the expectancy in HIV patients (Bobbarala*et al.*, 2009). The PI's from the leaves of *Coccinia grandis*found to exhibit a high degree of inhibition of growth in *K.pneuminiae* and *A.flavus*(Satheesh and Murugan 2011). The serine protease inhibitors are characterized from leguminosae, solanaceae and graminae families (Gracias- Olmedo*et al.*, 1987).

The serine protease inhibitors can be classified into13 structurally distinct families based on the source. Six families of SPI are of mammalian or microbial origin they are Hirudin family, Bovine Pancreatic Trypsin inhibitor (BPTI)family, the Kazal family, the Chelonianin family, the Streptomyces subtilisin inhibitor(SSI)family, Serpins family.

Seven families are of plant origin which include Cucurbit family, Bowman-birk family, the Cereal super family, the Patato serine protease inhibitorfamily, the Thaumatin family, the Kunitz-type family. In these the two best characterized families of plant serine PIs are the Kunitz-type and Bowman-Birk type inhibitors. These families differ from each other in mass, cysteine content and number of reactive sites (Richardson 1977). Structurally, both Kunitz and Bowman–Birk inhibitors lack a-helix, but vary in their mode of stability. Kunitz inhibitors are stabilized chiefly by hydrophobic interactions of short stretches of hydrogen bonded sheets (soybean Kunitz trypsin inhibitor) whereas the disulfide linkages in the Bowman–Birk inhibitors minimize their conformational entropy and enhance their stability (Ramasarma*et al*., 1995).

Markus Hartl *et al.*,(2011) identified four serine PIs (SPI) from *Solanum nigrum* and demonstrated that they differ substantially in substrate specificity, accumulation patterns, and their effect against different natural herbivorous insects.

Hirudin is a serine protease inhibitor isolated from the saliva of leeches (*Hirudo medicinalis*) and it is specific for thrombin, a serine protease involved in the blood coagulation (Rydel*et al.*, 1990).

Members of Bovine Pancreatic Trypsin inhibitor(BPTI) are stabilized by 3 disulfide bridges(Yu *et al*.,1995).It isa 58 amino acid residue protein consist of α-helix on the N-terminus, a highly twisted anti-parallel β-sheet and α-helix near the C-terminus. BPTI isolated from egg yolks there was no cross reactivity against alpha1-proteinase inhibitor (human and sheep), inter-alpha-trypsin inhibitor (human and sheep). And shows activity against lung and pancreas diseases (Melrose*et al.*, 2000).

Kazal family was identified by Kazal *et al*., in 1948. These are double headed inhibitors which inhibit trypsin and chymotrypsin simultaneously(Mistry*et al.*, 1997). Kazal isolated from potato (Solanum tuberosum) and tomato (Lycopersicon esculentum) shows activity against *P. infestans*(Miaoying Tian*et al*.,2005).

Secretory leukocyte protease inhibitor (SLPI) and R-Elafin are the most familiar members of Chelonianin PIs (Francart*et al*., 1997). It is a double headed PI with7 disulphide bonds and inhibiting trypsin and elatase(Stergios Doumas*et al*.,(2005)). SLPI isolated from plant sources like soybean and lima bean, potatoes (Melrose *et al*., 2000).

Streptomycessubtilisin inhibitor(SSI)family specifically inhibits alkaline protease such as subtilisin although it also weakly inhibits trypsin and chymotrypsin(Mitsui*et al.*,1979).

Serpin family is the largest and the most widespread super family of PIswith 60 members. These inhibitors widely occur in all type of organisms including viruses, bacteria, plant and animals (Irving*et al*., 2002). They inhibit serine protease such as trypsin by irreversible inhibition so called as suicidal inhibitors (Janciauskiene 2001). Barley (*Hordeum vulgare*) is a potent inhibitor of trypsin and chymotrypsin with over lapping reactive sites (Kervinen*et al*., 1999) and active against *Agrotis ipsilon*(Carboneroo*et al*., 1993)and *Spodoptura litura*(Ussuf*et al*.,2001).

An inhibitor from Cucurbit familywas isolatedfrom *Momordica charantia* inhibitor (MCI-3)a trypsin inhibitor form cucurbitaceae. This protease Inhibitor involved in the blood coagulation (Zeng*et al*.,1998).

Bowman- Birk PIs are named after DE Bowman and Y Birk who were first to identify and characterizeda member of this family from soybean (*Glycine max*). This PI was composed of proteins with 7 S-S bridges (Werner and Wemmer 1991). Trypsin and chymotrypsin inhibitors from soy and bean seeds exhibited inhibition against proteinases secreted by *Fusarium solane*(Benken *et al*., 1976).The Bowman– Birk type inhibitors have M wt 8–10 kDa as well as high cysteine content and two reactive sites (Trexler and Banyai 2001).These are found in legume seeds like soybean and pea (Ferrasonet al.,1997). These inhibitors interact independently, but simultaneously with two proteases, which may be same (or) different (Raj*et al.*, 2002). The first reactive site in these inhibitors is usually specific for trypsin, chymotrypsin and elastase (Qi*et al*.,2005). The active site configuration in these inhibitors is stabilized by the presence of seven conserved disulfide bonds 9(Lin*et al.*, 1993). Buck wheat antifungal peptide suppresses the proliferation of the various tumor cells that are of hepatoma, leukemia and breast cancer(Noemí Eiró*et al.*, 2013). The Trypsin inhibitors of Bowmann Birk family present in the developing seed and suppress the growth of soil born pathogen infection(Edwin and NG2007). SFTI from sunflower seed which is composed of 14 amino acid (Hernandez*et al*., (2000)) and three macro cyclic trypsin inhibitors from squash seeds composed of 34 amino acids (Korsinezky*et al.*, 2001).A short peptide of serine peptidase inhibitors ORB-2, ORB-2k are likely Bowman - Birk inhibitor which acts as antimicrobial agent contain multi cationic residue that are easily destroyed by trypsin like proteases. This ORB was purified from skin secretions of the frog *O. graham* which has strong antimicrobial activity but weak inhibitor activity against trypsin

Cereal super family are small group of SPIs extracted from cereals such as wheat, barley, maize and ragi (Campos and Richarson (1983)). They show inhibition against Trypsin and hageman factor and also inhibits α – amylase. A trypsin inhibitor from wheat kernel also elicits a potent antifungal effect (Chilosi*et al*., 2000).Soap Nut trypsin inhibitor from *Sapindus trifoliatus (*soap nut*)* shows antifungal activity against dermatophytic fungi *Trichophyton rubrum* and *Malassezia furfur* which are causative agents of dandruff. It has also exhibited antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris* and *Escherichia coli* (Rachel*et al.*, 2013).

Potato Serine Protease Inhibitor (PSPI)is heterodimeric. The crystal structure of PSPI represents the first heterodimeric double-headed Kunitz-type serine protease inhibitor structure determined. PSPI has a β-trefoil fold and based on the structure, two reactive site loops bearing residues Phe75 and Lys95 have been identified (Meulenbroek*et al.*,

2012). These are sub-divided into two families 1. Potato inhibitor type-I (PI-I)family 2.Potato inhibitor type-II (PI-2)family.Expression of Potato PI-I and PI-II genes in transgenic petunia, birch and lettuce plants showed resistance to *Lepidopteran* and *Orthopteran* insects and *M. sexta*. Transgenic tobacco plants contain chymotrypsin inhibitor. This gene isolated from potato which enhance *Chryodeixis eriosoma*(Ussuf et al.,2001).

Potato inhibitor type-I(PI-I)inhibitors have been extracted from potato tubers (Ryan and Balls 1962), tomato fruit (Wingate*et al*., 1989), squash phloem exudates (Murray and Christeller1995) and in tomato leaves in response to wounding (Lee*et al*., 1986). Each subunit of this PI contain one S-S bond and is double headedagainst tyrpsin and α -chymotrypsin.

Potato inhibitor II (PI-II) inhibitorsare found in leaves, flowers, fruit and phloem of Solanaceaous species (Pearce*et al*., (1993)) PI-II was also purified from tomato (Taylor*et al*., 1993). Trypsin and chymotrypsin inhibitors from potato tubers exhibited inhibition against proteinases secreted by *Fusarium solane*(Benken*et al*., 1976). PI-II showed inhibition against Trypsin, α - chymotrypsin and elastase. In Arabidopsis, Maize, Rice and *S. altissima* derived SPI shows strong antimicrobial activity towards *S. exigua* by affecting the gut protease (Zavala*et al*., 2004). Rice actin I gene and wound inducible PI-II promoter expressed in transgenic rice acts against pink stem borer (*Sesamia inferens*). Sweet potato trypsin inhibitor gene expressed in transgenic tobacco and cauliflower plants resulted in enhancing resistance against *S. litura*(Ussuf*et al*.,2001).

Kunitz- type family is mostly active against serine proteases but may also inhibit other proteases (Laing 2002). Kunitz-type inhibitors are proteins of M wt 20 kDa, with low cysteine content and constitutes only a single reactive site (Richardson 1991). These PIs were described in legumes, cereals and solanaceous species(Chye*et al*., 2006). These PIs are produced under stress and have been found in potato tubers (*S. tuberosum*) (Ledoigt*et al*., 2006). The members of this family are mostly active against serine proteases and they inhibit Trypsin, chymotrypsin and subtilisin (Park*et al*., 2005). A 20.5 kDa Kunitz-type trypsin inhibitor with antifungal activity has been reported from the roots of punce ginseng (*Pseudostellaria heterophylla*) (Wang and Ng2006). In 2004 Macedo *et al*. ,reportedthat Kunitz type PI from *Prosopis juliflora* shows activity against trypsin and chymotrypsin and exerts insecticide effects against *C. maculatus* larvae by blocking the enzymes in the insect's digestive system. WSG (*Withania somnifera* glycoprotein) isolated from *W. somnifera* root tubers revealed (protease inhibitor) antimicrobial activity against few bacterial and phytopathogenic fungi(Mahesh and Satish 2008). WSG also provided a fungi stastic effect by inhibiting spore germination and hyphal growth in the tested fungi.

Thaumatin family inhibits specifically α- amylase but in some cases trypsin also. It is a group of monomeric proteins with 8 S-S bonds (Franco*et al*, 2002).

Inducible serine protease inhibitors (ISPI)are three types, they are ISPI-1 ISPI-2 ISPI-3 isolated from the hemolymph of wax moth larvae (*Galleria mellonella*) These inhibitors are active against various serine proteases including trypsin and toxic proteases released by the entimopathogenic fungus *Metahizium anisopliae* The aminoacid sequence of the ISPI-2 is similar to kunitz- type protease inhibitors.

Cysteine Protease Inhibitors (CPIs)

Cysteine super family comprises both eukaryotic and prokaryotic cysteine protease inhibitors(Rawlings and Barrett (1990)). Human cystatins C,D and S, rat cystatins A and S, chicken cystatins and oryza cystatins have been reported to inhibit the replication of certain viruses and bacteria (Tollin*et al*., 2005). Targeting papain family cysteine

protease is one of the novel stratagies in the development chemotheraphy of many diseases. Novel cysteine protease inhibitor derived from 1-pyridylimidazo pyridine representing pharmologically important class of compounds are being reported for first time by Khan*et al.,* in 2013. These compounds were specific inhibitors of cysteine protease. Pepstatin didn't show inhibition against other proteases like serine aspartic or metallo proteases. The proteolytic activity of extracts of adult western flower thrips (WFTs) (*Frankliniella occidentalis),* highly polyphagous insect (Jensen 2000) has an optimum PH 3.5 and is nearly completely inhibited by protease inhibitors (PIs) specific for cysteine proteinases are predominant in WFT digestive tract was subsequently supported by the gradual reduction in WFT oviposition rate when purified potato cystatin and equistatin were fed to adult females in combination with a protein rich pollen diet(Annadana*et al*., 2002).

The class of cysteine PI is also called as cystatin super family, this super family excludes the members belonging to the kunitz- type inhibitor and the clitocypin family. In animals cysteine protease Inhibitors can be classified into 3, the stefin, cystatin and kininogen families according to their molecular mass and the presence and absence of cysteine residue.

Stefin family group of monomeric proteins are with 12 kDa molecular weight without cysteine residue (Pernas*et al*., 1998). It is a potential competitive inhibitor for cysteine protease such as papain, bromelain and cathepsin-H (Katumuna and Kominami 1983).

Cystatin family have 2 disulphide bonds located towards C- terminal end of molecule with molecular mass of 11 kDa (Turk and Bode 1991). Cathelin-like protein shares homology with the cystatin family of cysteine protease inhibitors (Sorensen*et al*., 2001). The human cathelin-like domain exhibitsantibacterial activity against pathogens including *Escherichia coli*and methicillin-resistant *Staphylococcus aureus* and also inhibit bacterial growth and cysteine proteinase- mediated tissue damage(Mohamed Zaiou*et al*., 2003). Cathelicidin which is secreted in wound fluid of human skin are antimicrobial peptides and are investigated extensively. The structure of this protein domain contains 4 cystiene molecules with two disulphide bonds. This recombinant cathelicidin inhibits the growth of *S.epidemidis* (Mohamed Zaiou*et al*., 2003). Cysteine PI from rice was introduced in transgenic tobacco, potato, oil seeds, rape and cotton suppressed the activity of beetle feeding and also acts against poty virus, tobacco etch virus and potato virus Y. Corn cystatin with AMV 35 promoter gene in rice showed against insect gut protease of *Sitiophilus zeamais*(Ussuf2001).

Kininogen family inhibitors are glycoproteins and subdivided into 3 groups. High molecular weight kininogen (HMW)(120 kDa) L-kininogen(68 kDa) and T-kininogen(68 kDa) (Turk and Bode 1991). Its characteristics are similar to stefin and cystatin families (*Turk et al.,*(1997)). They are identified in rice, maize (Abe*et al*., 1987), apple (Ryan*et al*., 1998) soybean (Hines*et al.,* 1991) and Carnation leaves (Kim*et al*., 1999).

Multicystatin family contain multi domains identified in potato tubers (85 kDa), tomato leaves and sunflower seeds (Wu & Haard2000).

Kunitz type family shows the sequence homology with kunitz-type serine protease inhibitors. And inhibit the cysteine proteinase papain (Krizaj *et al*., 1993).

Chitocypin family is a recently discovered cysteine protease inhibitor isolated from the mushroom (*clitocybe neburalis)*. This do not show any sequence homology. It shows inhibition against papain, cathapsin-2 and B and Bromelain but is inactive against trypsin(Kidric*et al*., 2002).

The inhibitor properties of the purified inhibitors, kininogen domain 3(K), stefin A(A), cystatin C(C), potato cystatin (P), and equstatin (EI) were compared with the synthetic cystein PI E-64 in WFT homogenate as enzyme source at high concentrations. Most inhibitors efficiently inhibited the proteolytic activity of the enzyme. But the proto cystatin inhibited only 60% activity so it was classified as a weak inhibitor. Equistatin was a strong inhibitor (Nikolay S. Outchkourov *et al*., 2004). The strong activity of equistatin shows activity against both cysteine and aspartic proteases.

Metallo Carboxyprotease Inhibitors (MCPIS)

Metallo carboxyprotease inhibitors from plants can be distinguished intotwo families. The Metallocarboxy peptidase inhibitor family and a cathepsin-D inhibitor family.Metallocarboxy peptidase inhibitors isolated from potato and tomato plants (Rancor and Ryan(1968))and a cathepsin-D inhibitor isisolated from potato. These inhibitors are small protein inhibitors consisting of 38-39 amino acid with 3 S-S bonds and the molecular weight of 42kDa (Hass and Hermodson 1981). These inhibitors inhibits by competitively a broad spectrum of carboxy peptidases from both animals and microorganism but not serine carboxy petidases from yeast and plant (Havkioja and Neuvonen1985). The inhibitors that bind to metallo carboxypeptideses identified in solanaceaous plants and organisms like *Hirudo medicinalis*, in the blood tick *Rhipicephalus bursa*, in the intestine parasite round warm, *Ascaris suum*, in rat and in human tissue (Arolas*et al*., 2005). These inhibitors inhibits the activity of thermolysin, matrilysin, neutrophil, collagenase, interstitial collagenase, atrolysin C, stromelysin, carboxypeptidase A, and TNF – α convertase. The potato carboxypeptidase inhibitor (CPI) extracted from *Solanum tuberosum*inhibits thermolysin metallocarboxypeptidase.

Similar to other enzymes MMPs are regulated by the naturally occurring inhibitors called tissue inhibitors of metalloproteinases (TIMPs)(Tency*et al*., 2012). To date 4 TIMPs have been described they were TIMP-1, TIMP-2, TIMP-3, and TIMP-4. These inhibitors are tight binding proteins carrying 20 to 30 kDa molecular mass which are secreted proteins found at all cell surface in association with membrane bound proteins. TIMPS are expressed in tissues and in many cell types, and its expression is regulated during development and tissue remodeling (Fabrizio Bruschi and Barbara Pinto 2013). And also have anti-angiogenetic and anti-apoptotic effects(Guedez1998). In addition to natural inhibitors some synthetic inhibitors are also available to regulate the activity of the MMPs such as o-phenanthroline (phen), hydroxamates and tetracycline type antibiotic. These share the common characteristics of chelating Zn^{2+}(Brew2000)

There are three components of MMP inhibitors are – Zinc binding group (ZBG), the peptide backbone and the pocket occupying side chain. The requirements for a molecule to be an effective inhibitor of the MMP class of enzymes are (i) a functional group (eg: carboxylic acid, hydroxamic acid and sulfhydryl etc) capable of chelating the active site Zinc (II) ions. (ii) at least one functional group which provides hydrogen bond interaction with enzyme backbone and (iii) one or more side chains which undergo vander waals interactions with enzyme substrates (Lenart 2013).

A parasitic infection caused by Plasmodium, African *Trypanosoma*, *T.gondii*, *T.solium*, *A.contonensis* may involve the Central Nervous System, is represented by increased levels of MMPs, which are induced either directly or indirectly by regulating cytokine levels. These MMPs are regulated by the TIMPs and suppress the activity and control the diseases. TIMPs are involved in the suppression of inflammatory bowel disease, colorectal cancer(László Herszényi2012), viral hepatitis – B and C, alcoholic liver cirrhosis, cervical infection by human papiloma virus, cervical cancer, breast cancer, pancreatitis and gastric infections.

CONCLUSIONS

Protease inhibitors play a major role in the different regulatory reactions and control the different pathogenic process of human diseases such as arthritis, pancreatitis, hepatitis, cancer, AIDS, thrombosis, emphysema, blood pressure, muscular dystrophy, used as bio insecticides and even act as antimicrobial agents as they are capable of inhibiting the growth of different microorganisms like bacteria, virus and fungi. Transgenic plants producedbyrDNA technology, where in protease inhibitor geneswere transplanted and these transgenic plants have potentially developed resistance towards insects by enhancing their metabolic reactions. Currently over 60 distinct families of protease inhibitors have been recognized. These inhibitors are reversibly interacting with enzyme targets, forming stable complexes by influencing their catalytic activities in competitive and non-competitive ways. The capability of inhibiting the proteins that are responsible for many dreadful diseases have proved the protease inhibitors as an most effective agent in curing diseases and hence these protein inhibitors have need to be study well and further provides an excellent area of research.

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